The Effect of Splenectomy on Platelet Formation and Megakaryocyte DNA Content in Rats

By Gunnar Tanum, Anne Sørstevold, and Erling Jakobsen

The DNA content of rat bone marrow megakaryocytes (MK) was studied by Feulgen photometry following splenectomy and sham operation, respectively. The DNA measurements were preceded by acetylcholinesterase staining for identification of the 2N–8N MK. The number of 2N–8N MK increased to maximum values about 4 days after splenectomy, while the number of 16N–64N MK increased to maximum values about 4 days following both splenectomy and sham operation. However, the changes were somewhat more pronounced following splenectomy than sham operation. The total MK number did not change significantly. Platelet production, measured by 35S incorporation into platelets, increased during the first 2 days and remained high for 6–7 days, increasing the platelet production at days 2, 3, 4, 5, 6, 7, 11, and 30 following surgery. The number and DNA content of bone marrow MK were recorded in all animals, except in those sacrificed on days 5 and 6.

A RISE IN THE BLOOD platelet number following splenectomy has been reported in several species of mammals, including rat and man. General surgery, dependent on its extensiveness, also leads to a rise in blood platelet number, but with a lower number and a shorter duration than after splenectomy. The mechanism by which the spleen exerts an effect on the number of peripheral platelets has not been established. As reviewed by Rolovic and Baldini, one hypothesis postulates negative feedback on platelet production of a splenic humoral factor. Another hypothesis is that the spleen forms a platelet pool in equilibrium with general circulation. Both hypotheses have some experimental and/or clinical support. In the present study, platelet number, platelet production, MK number, and the DNA values of bone marrow megakaryocytes (MK) were examined in splectomized and sham-operated rats in order to clarify whether or not splenectomy changes megakaryocytopoiesis and platelet production and whether there exists a fundamental difference between splenectomy and sham operation in this respect.

MATERIALS AND METHODS

Forty-eight male inbred Lewis rats (supplied by Mjøllegaard-Hansen Avlslab A/S, Denmark), 2-2.5 mo old, were used for the study. The animals were kept under controlled environmental conditions. Splenectomy was carried out on 24 animals under sterile conditions and ether anesthesia, through a transversal incision in the left hypochondrium of the abdomen after ligation of the blood vessels. The other 24 animals were sham operated by holding the spleen outside the abdomen for the same period of time as needed for splenectomy. The spleen was then replaced in the peritoneal cavity. The peritoneum, muscles, and skin were closed by silk sutures. The animals behaved normally after the operation, and postmortem inspection of the abdomen showed no signs of inflammation or bleeding. Five unoperated animals were used as normal controls.

Groups of three splectomized and three sham-operated animals were sacrificed and examined for their blood platelet number and platelet counts. All values were about normal 30 days after surgery, except for a minor thrombocytosis following splenectomy. The early, highly significant thrombocytosis, following both splenectomy and general surgery, is caused by increased production of platelets due to the surgical trauma. This is caused by a direct action on bone marrow MK by transforming 2N–8N MK into higher ploidy classes. Lack of splenic platelet pooling may influence the grade and duration of the early thrombocytosis after splenectomy. The late, long-lasting, minor thrombocytosis, which occurs after splenectomy but not after sham operation, can be explained by the removal of the splenic platelet pool.

Platelet Counts

Under ether anesthesia, 0.1 ml blood was collected from a femoral vein and mixed with 1 ml Isoton II (Coulter Electronics Ltd., Luton, England). The platelets were counted by a Coulter Counter (Coulter Counter S+, Coulter Electronic, Inc., Hialeah, FL).

Platelet Production

The platelet production was determined by measuring the incorporation of 35S-sodium sulfate into the platelets, as described by Dziewiatkowski and Odell et al. The platelets were labeled in vivo by an intraperitoneal injection of 8.3 megabequerel 35S-sodium sulfate dissolved in sterile 0.9% NaCl, given 42 hr before the animals were sacrificed. Preliminary studies showed that 35S incorporation into platelets did not differ significantly whether the 35S was given intraperitoneally or intravenously to the operated rats. This indicates that the surgical procedures performed in the present study did not significantly affect the absorption of 35S from the peritoneal cavity. Blood was collected from the aorta after laparotomy. Two milliliters platelet-rich plasma was washed in 1% ammonium oxalate and 0.9% NaCl, resuspended in Lumagel (Lumac System AG, Basel, Switzerland), and the 35S activity was counted in a liquid scintillation system (Mark III, Searle Analytic Inc., Des Plaines, IL). The 35S incorporation into platelets was calculated as a percentage of the total injected 35S dose. In pilot studies, plasma radioactivity was measured at various intervals following 35S injection to monitor for the possibility of an abnormality in sulfate metabolism in the operated animals compared to the controls. The curves for plasma radioactivity were about parallel, indicating that sulfate metabolism was similar for both groups.

From the Department of Haematology and Lymphology, Norwegian Radium Hospital, Oslo, Norway.

Submitted November 4, 1982; accepted September 19, 1983.

Address reprint requests to Dr. Gunnar Tanum, Department of Haematology and Lymphology, Norwegian Radium Hospital, Montebello, Oslo 3, Norway.

© 1984 by Grune & Stratton, Inc.

0006-4971/84/6303-0015$01.00/0

Blood, Vol. 63, No. 3 (March), 1984: pp. 593–597
MK Number and DNA Content

The method for measuring number and DNA content of rat bone marrow MK has been thoroughly discussed previously, especially with respect to the reliability of the method and its implications compared to previous MK studies. Briefly, the bone marrow was completely removed from both femur diaphyses. All MK on one smear made from 1.2 μl bone marrow from each animal were identified by S-acetylthiocholiniodide staining. In rodents, this stain binds to acetylcholinesterase and colors the platelets and MK brown, including the low ploidy MK, which are otherwise difficult to identify. Other bone marrow cells remain unstained. All MK on the smear were identified and numbered, and the MK number per microliter bone marrow could thus be calculated. The position of the MK was recorded by a grade system. For photometry, the smear was decolorized, as described by Jackson, and restained with Feulgen stain. In each smear, a sample of 101 MK was examined for the DNA content. This sample was randomly selected by drawing numbers allotted to all MK in the smear. Photometry was performed by a scanning microspectrophotometer (SMPO Zeiss, Oberkochen, W. Germany). A wavelength of 570 nm was used, corresponding to the stained component's absorption maximum, with a ×100 objective immersed in glycerol. For 2N reference value, 25 segmented granulocytes from each smear were measured. The DNA values of the various ploidy classes were calculated on the basis of the observed granulocyte diploid value. Each ploidy class was defined according to an internationally recognized convention, whereby all MK are placed into ploidy classes without any interploidy compartment.

RESULTS

In the controls, the number of blood platelets was $0.738 \pm 0.027 \times 10^6/\mu l$ and the $^{35}$S incorporation into platelets was $4.9\% \pm 0.3\% \times 10^{-3}$ of the injected dose. In the operated animals, the blood platelet counts increased significantly after 2 days, and at day 6, reached a maximum of about 190% and 140% of normal values following splenectomy and sham operation, respectively (Fig. 1A). At day 30, the mean blood platelet number was within normal range following sham operation, but about 15% above normal ($p < 0.05$) following splenectomy. Also, the $^{35}$S incorporation into platelets rapidly increased after surgery and was about 220% and 200% of normal at day 2 following splenectomy and sham operation, respectively (Fig. 1B). One day after normalizing at days 11 and 7, respectively. The curve for platelet production had much the same shape as the curve for platelet counts in both operated groups. There was a transient drop in both platelet counts and platelet production in both operated groups around days 2–4.

There were no significant changes in the total MK number following splenectomy or sham operation (Fig. 1C). Figure 1 presents the variations in MK number within the ploidy classes of 3,629 MK recorded. The number of MK within the 2N–4N compartment decreased to about 10% of normal 4 days after splenectomy ($p < 0.05$), but did not change significantly following sham operation. The number of 8N MK dropped to a minimum of about 10% and 50% of normal 4 days following splenectomy and sham operation, respectively ($p < 0.01$). At the same time, the number of MK within the 16N–64N compartment increased to about 200% and 150% of normal after splenectomy and sham operation, respectively ($p < 0.05$). The number of MK within all ploidy classes was about normal 30 days following surgery (Fig. 2, A and B).

DISCUSSION

In accordance with previous studies, we found that blood platelet counts and platelet production increased significantly during the first days following both splenectomy and sham operation. In the present study, no significant increase in MK number could be recorded following splenectomy. The increased MK
number found by other investigators \(27\) can be explained by the increased number of mature cells. The corresponding decrease of small MK (Fig. 2, A and B) has probably been overlooked in previous methods.

Following splenectomy, the number of 2N–4N and 8N MK dropped within 2 days and stayed low for at least 9 days, while the number of 16N–64N MK increased during the first 3–4 days (Fig. 2A). This indicates that the 2N–8N MK develop into 16N–64N MK faster than normal, whereas the influx of cells from the MK precursor compartment is not correspondingly increased. This is further supported by the fact that the total MK number stayed normal. One theoretical, but very unlikely, explanation is that the postsplenectomy thrombocytosis is caused by elimination of a splenic humoral factor inhibiting megakaryocyte maturation and/or platelet delivery.\(^2\)\(^,\)\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\)\(^,\)\(^11\)\(^,\)\(^12\) Others explain this thrombocytosis by the removal of an exchangeable splenic platelet pool, which is in constant equilibrium with the blood platelets in general circulation.\(^1\)\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\)\(^,\)\(^11\)\(^,\)\(^12\) This pool normally contains 12%–15% of the total platelet mass in rats.\(^12\)

Following general surgery, the blood platelets are thought to be squeezed out of the spleen by adrenergic stimulation.\(^2\)\(^9\)\(^,\)\(^30\)\(^,\)\(^31\) However, Ebbe et al. concluded that MK maturation rate is increased following surgery.\(^3\)\(^5\) This is a well-known mechanism to increase platelet production.\(^2\)\(^9\) One explanation may be that tissue damage in general stimulates the MK by acting on specific humoral agents. This is in accordance with the studies of Odell et al., who found that powdered glass and egg albumin injected subcutaneously in rats increased the number of blood platelets.\(^27\)

The platelet production increased during the first days after surgery, but was about normal for both groups at days 11 and 30. The increased platelet counts at day 11 in splenectomized animals, together with normal platelet production at this time, can be explained by the high platelet production within this group 4 days previously. The platelet lifespan in rats is 4–5 days.\(^2\)\(^9\)\(^,\)\(^30\)\(^,\)\(^32\) The lack of splenic platelet pooling might also have an effect. According to Shulman et al., the spleen contains comparatively more young blood platelets than old ones.\(^3\)\(^6\) Thirty days after splenectomy, the blood platelet counts still stayed high for the
splenectomized animals, but were normal for the sham-operated animals, whereas the platelet production was about normal for both groups (Fig. 1, A and B). Thus, the thrombocytosis at day 30 after splenectomy cannot be caused by lack of a splenic inhibitory factor on platelet production, and the most probable explanation is that this is an effect caused by the removal of a splenic pool. The present study does not support the hypothesis of a splenic inhibitory factor.

The variations in blood platelet number, platelet production, total MK number, and MK number within different ploidy classes followed the same pattern for the sham-operated as for the splenectomized animals during the first week after surgery. However, the changes were more pronounced following splenectomy compared to sham operation. Splenectomy was performed with ligation of large blood vessels, and hence, was a greater surgical trauma than sham operation, where no resection was performed. It has previously been shown that the degree of thrombocytosis corresponds to the extensiveness of the operating trauma.7

The present study shows that the early, highly significant thrombocytosis, both following splenectomy and general surgery, is caused by increased production of platelets due to the surgical trauma. This is caused by a direct action on bone marrow MK by transforming 2N–8N MK into higher ploidy classes, but without any significant increase in total MK number. These effects on MK may be mediated by thrombopoietin, which is known to induce much the same changes as were observed in the present study.37 Lack of splenetic platelet pooling may influence the grade and duration of the early thrombocytosis after splenectomy. The late, long-lasting, minor thrombocytosis, which occurs after splenectomy but not after sham operation, can be explained by the removal of the splenic platelet pool.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical help given by A. Skjien and the assistance of the Department of Tissue Culture, Norwegian Radium Hospital.

REFERENCES

8. Dziewiatkowski DD: Rate of excretion of radioactive sulphur and its concentration in some tissues of the rat after intraperitoneal administration of labeled sodium sulphate. J Biol Chem 178:197, 1949
The effect of splenectomy on platelet formation and megakaryocyte DNA content in rats

G Tanum, A Sonstevold and E Jakobsen